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Ultramicroelectrode Sensors and Detectors. Considerations of the Stability, Sensitivity, Reproducibility, and Mechanism of Ion Transport in Gas Phase Chromatography and in High Performance Liquid Phase Chromatography

by

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ABSTRACT

We describe in this paper the use of ultramicroelectrodes as sensitive sensors and detectors in the gas phase and in liquid solutions of high resistance; particularly aspects regarding the sensitivity, stability, and reproducibility of of the devices. The advantages of these detectors can be improved notably by appropriate choice of method of pretreatment of the electrode surface. The surface separating the working ultramicroelectrode from the secondary/reference electrode provides the medium for ionic conduction for the cell. The mechanism for ion conduction involves proton migration and diffusion across the surface of the separator.

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ULTRAMICROELECTRODE SENSORS AND DETECTORS

CONSIDERATIONS OF THE STABILITY, SENSITIVITY, REPRODUCIBILITY, AND MECHANISM OF ION TRANSPORT IN GAS PHASE CHROMATOGRAPHY AND IN HIGH PERFORMANCE LIQUID PHASE CHROMATOGRAPHY

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ABSTRACT

We describe in this paper the use of ultramicroelectrodes as sensitive sensors and detectors in the gas phase and in liquid solutions of high resistance; particularly aspects regarding the sensitivity, stability, and reproducibility of ~~of~~ the devices. The advantages of these detectors can be improved notably by appropriate choice of method of pretreatment of the electrode surface. The surface separating the working ultramicroelectrode from the secondary/reference electrode provides the medium for ionic conduction for the cell. The mechanism for ion conduction involves proton migration and diffusion across the surface of the separator.

INTRODUCTION

Ultramicroelectrodes have found new and interesting applications as sensitive electrochemical sensors and detectors in a variety of media [1–4]. The improved analytical sensitivity observed at ultramicroelectrodes (compared to electrodes with characteristic dimensions larger than about 25 μm) is due to the increase in the relative contribution of faradaic to nonfaradaic currents as the characteristic size of the electrode is decreased [5]. At very small electrodes, electrochemical measurements in the absence of added supporting electrolyte is possible [6–8]; the number

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1

of ions/dipoles required to charge the double layer of a low area electrode is small so that impurities or ions resulting from to autoprotolysis of the solvent are sufficient. Further, the small currents ($< 10^{-10}$ A typically in dilute solutions) give rise to negligible ohmic corrections to the applied potential; this correction can be predicted analytically in some circumstances. Electrochemical measurements in highly resistive media are therefore straightforward at ultramicroelectrodes [9], and can be made at higher potentials that are possible in conventional systems where the limiting potential is due to electrolysis of the added supporting electrolyte [10,11]. The small currents flowing in the cell also eliminate the need for potentiostats since the counter electrode potential remains essentially constant during the experiment. System electronic noise is therefore reduced. Typical measurements are the made with two elecetrodes, a wave form generator, and a picoammeter [12].

The present work concerns the behavior of ultramicroelectrodes in gas phase measurements, particularly in gas chromatographic applications, and in high performance liquid chromatography. It was suggested in an earlier report [12] that the electrochemical reactions of species in the gas phase at ultramicroelectrodes are coupled to ion migration across the surface of the insulator separating the two electrodes. Evidence for this process and the role of protons in the ion transport mechanism are discussed.

The use of electrochemical detection in high performance liquid chromatography is expanding rapidly [13]. The requirement of supporting electrolytes in the liquid phase has generally precluded the use of electrochemical detection in pure liquid chromatographic phases; the solvent often must be a non-polar organic solvent. The same experimental requirements are generally required for flow injection analysis experiments. We demonstrate herein the utility of ultramicroelectrodes for chemcical sensing and detection under these circumstances.

EXPERIMENTAL

An ultramicroelectrode gas phase detector assembly is shown in Fig. 1. A detailed line drawing of the electrode assembly itself is presented in ref. 1. The working electrode used in this work for both the gas phase and high performance liquid phase chromatography experiments consisted of a 25 μm diameter platinum wire (Johnson Matthey, Inc.), lead soldered to the end of an 18 ga. copper wire. The assembly was then inserted into a quartz tube (0.5–0.7 mm i.d. at the tip) until a few mm of wire protruded from the tip of the tube. The tube was previously coated with a thick film of palladium metal on the outside surface to a length of about 2 cm from the tip. This coating was prepared by thermal reduction of Liquid Bright Palladium (Englehard Industries, Inc.) organnometallic at 625°C for 1 h in a tube furnace. Electrical contact to the palladium coating was made by lead soldering to it a fine insulated copper wire. The platinum ultramicroelectrode sealed in the glass tube with epoxy resin, and when dry was polished with alumina. A wave form generator (Hi Tek Instruments, Model PPR1) was used to control the potential. The currents were measured with a digital picoammeter (Keithley model 617), placed in

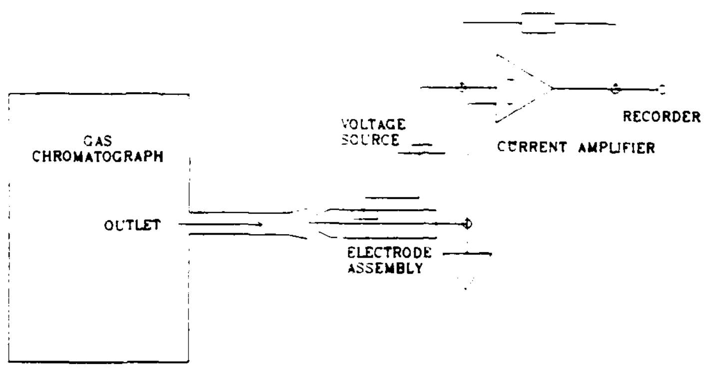


Fig. 1. Schematic diagram of the ultramicroelectrode chromatographic detector system.

series with the wave form generator and the electrochemical cell.

To reduce capacitatively coupled noise, the ultramicroelectrode assembly was positioned inside an aluminum Faraday cage. The gas chromatograph was a Hewlett-Packard Model 700. The gas outlet port was connected to the ultramicroelectrode detector by means of a section of Teflon tube. The tip of the ultramicroelectrode was inserted inside the Teflon tube which was vented near the ultramicroelectrode tip. A dual pen strip chart recorder (Omniscribe, Houston Instruments. Inc.) was used for the simultaneous recording of the response of the ultramicroelectrode and the thermal conductivity detector (Hewlett-Packard Model 4-4188). The reverse phase column used in all of the experiments described herein was Silicone OV-17 10% on ChromasorbW. The carrier gas was helium which was maintained at a constant flow rate of 30 ml/min. Tetra-n-butylammonium tetrafluoroborate (Chem.-Biochem. Research, Inc.) was used received. Reagent grade solvents were used.

For high performance liquid chromatographic studies, the DNA bases thymine, adenine, cytocine, uracil, and guanine (Aldrich Chemical) were used. The chromatograph consisted of a Valco 6-way electric valve, a Waters 6000A pump, and a 3.5 cm $\times$ 0.5 mm octadecylsilane reverse phase capillary column. The ultramicroelectrode detector was mounted on the end of the column so that the effluent passed across the tip.

RESULTS AND DISCUSSION

The initial experiments were intended to demonstrate the selectivity and relative sensitivity of the ultramicroelectrode in gas phase detection. The injection port was at a temperature of 170 °C and the column at 80 °C. The applied potential was +3.0 V vs. the palladium pseudoreference electrode. Table 1 shows the responses measured for injections of 1 μl of the various analytes. The first column in the table lists the ultramicroelectrode responses for the electrode prepared as described in the

TABLE 1

Response of the platinum ultramicroelectrode detector [a]

Compound	Response for electrode as prepared pA	Response after treatment w/$TBABF_4$ pA [b]	Ratio $\frac{\text{column 2}}{\text{column 1}}$
Acetone	0.1180	12.66	107.0
Acetonitrile	10.19	13.940	1368
Aniline [c]	0.2000	11.350	56.750
Benzonitrile [c]	0.8670	191.0	220.0
Carbon tetrachloride	0.2500	0.3120	1.205
Cyclohexanone	16.51	1.125	68.57
Ethanol	565.0	89.540	158.5
Ethyl Acetate	0.5300	136.6	258.3
Isopropylamine	0.1620	8.340	51.33
Methylene chloride	3.650	620.0	169.5
Methylamine (40% in water)	−51.000	168.700	3.394
o-Xylene	0.1010	16.13	160.1
Toluene	0.1620	0.3402	2.102
Triethylamine	0.5080	2.140	4.215
1,2,4-Trimethylbenzene	0.0120	2.590	216.3

[a] Injection of 1 μl; solution approx. 0.01 M H_2O.

[b] The ultramicroelectrode was pretreated in a 0.3 M in a solution of $TBABF_4$ in CH_3CN for 2 min.

[c] Injection of 0.5 μl; solution approx. 0.01 M H_2O.

experimental section, while the second column gives the measured responses after the electrode had been treated with a 0.3 M tetra-n-butylammonium tetrafluoroborate ($TBABF_4$) solution in acetonitrile for 2 min. The electrode was allowed to equilibrate at the exit port of the chromatograph for about 20 min. Generally, an increase in the sensitivity of the electrode was observed when treated with $TBABF_4$, which for the epoxy resin used was superior to other common electrolytes. The response of this detector is quite reproducible over a period of 12 h of continuous use. If the electrode was not treated with $TBABF_4$ for two consecutive days, a few percent decrease in sensitivity occurs, and will continue to degrade. Excellent reproducibility is obtained (better than 1%) if the electrode is treated daily by the procedure described. The absolute value of the increase depended on the solvent used, and varied from 100 to 1000 in over the untreated electrode. If the electrode was treated with 0.2 M H_2SO_4 solution for 1 min, a further increase in sensitiveity could be is observed, but was not reproducible or constant, presumably due to evaporation and oxidation of the epoxy resin.

The following experiment was carried out in order to compare the sensitivity of the ultramicroelectrode detector to the thermal conductivity detector. The effluent was first detected by the thermal conductivity detector and then the ultramicroelectrode detector. The ultramicroelectrode was located 5 mm downstream of the thermal conductivity detector. The gas chromatrograph and the ultramicroelectrode detector were operated under the same conditions as described in the last experiment.

1 μl injections of dilute cyclohexanone solutions in acetone were analyzed with both detectors. The results are given in Table 2. Correlation coefficients and slopes of the response/injected concentration plots for the ultramicroelectrode and for the thermal conductivity detector are also listed. The linearity of the two detectors can be compared better by plotting the logarithm of the response vs. the logarithm of the injected concentration [14], as shown in Fig. 2. We measure correlation coefficients of 0.998 and 0.999 for the ultramicroelectrode and the thermal conductivity detector, respectively, in the concentration range from 15% to 0.025% (v/v) of cyclohexanone. The slope of these plots, defined as the linearity of the detector [14], are 1.18 for the ultramicroelectrode and 0.84 for the thermal conductivity detector. The ultramicroelectrode used in these measurements has a ripple fluctuation of 3 fA peak to peak. The background noise fluctuation of the thermal conductivity detector was ca. 2 μV peak to peak. Defining the detection limits as the concentration that gives an average signal three times larger than the background noise level, and using the values of the slope in Table 2, we obtain detection limits for cyclohexanone in acetone of 160 ppm when measured at the ultramicroelectrode and 600 ppm at the thermal conductivity detector. A similar analysis for aniline solutions in acetone indicated detection limits of 82 ppm at the ultramicroelectrode detector and 900 ppm for the thermal conductivity detector.

The dependence of the ultramicroelectrode response on the applied potential and the nature of the analyte was investigated. The ultramicroelectrode response for an injection of a solution of 50% toluene (v/v) in triethylamine was determined in the potential region between +0.6 and +3.0 V vs. the palladium reference electrode. The column and the carrier gas used were the same as described in the experimental

TABLE 2

Ultramicroelectrode and thermal conductivity detector responses for cyclohexanone solutions [a]

Injected concentration % cyclohexanone	Ultramicroelectrode response pA [b]	Thermal conductivity detector response mV [b]
8	4.47	0.8
5	2.6	0.54
3	1.351	0.36
1	0.345	0.126
0.5	0.166	0.07
0.25	0.095	0.046
0.1	0.028	0.0186
0.05	0.0153	-
Corr. coefficient [c]	0.997	0.998
Slope [c]	0.556 pA/%	0.100 mV/%
Detection limits [d]	160 ppm	600 ppm

[a] 1 μl solution injected.
[b] Peak height.
[c] From the plot of the response vs. the injected concentration for the two detectors.
[d] Three times the electronic noise level.

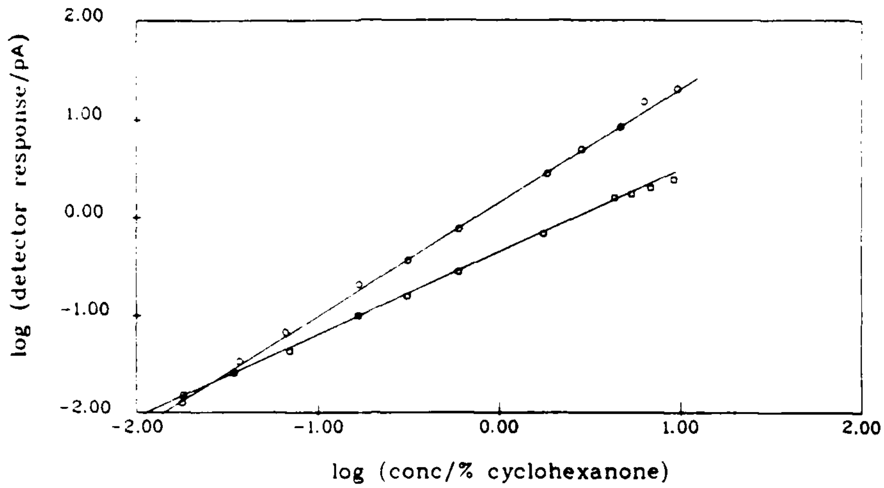


Fig. 2. Logarithm of ultramicroelectrode response vs. logarithm of cyclohexanone concentration (○), and log of thermal conductivity response vs. log of cyclohexanone concentration (□), for the injection of 1 μl of solution.

section. The injection port temperature was maintained at 120 °C and the column at 85 °C. Figure 3 shows the polariztion curves obtained for the two compounds 1 μl injections of the solution. There is a greater dependence on potential of the toluene response than for the triethylamine response. This leads to a crossing of the two voltammograms at lower potentials. The same experiment was performed with a 30% cyclohexanone solution (v/v) in triethylamine, between +0.8 and +7.0 V. The

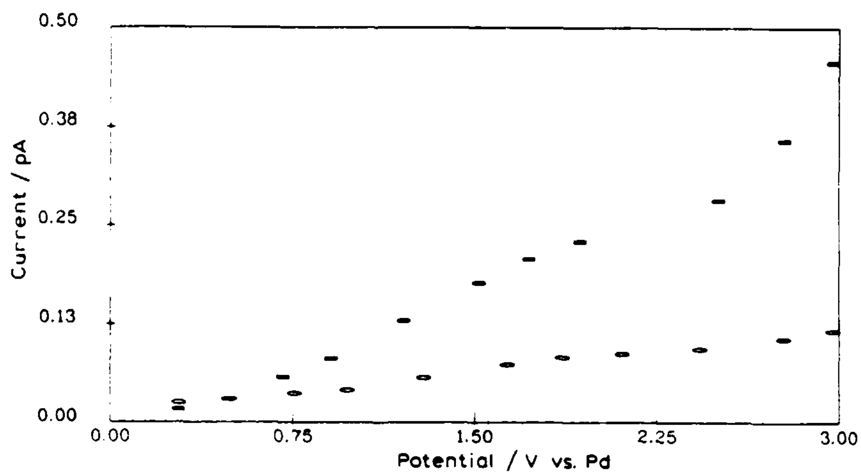


Fig. 3. Ultramicroelectrode response vs. potential applied for the injection of 1 μl of a solution 50% in toluene and 50% in triethylamine. (□) Toluene response, (○) triethylamine response.

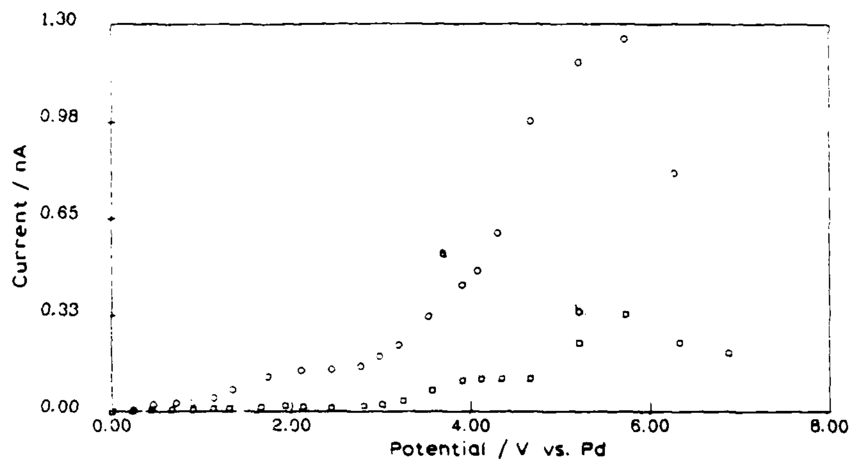
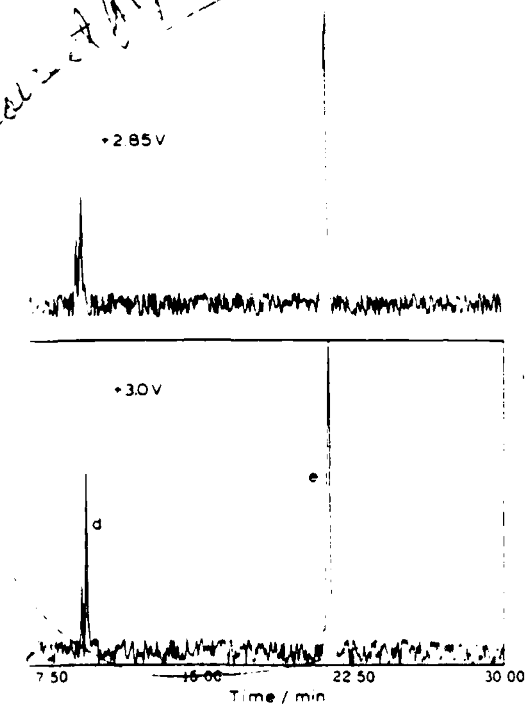


Fig. 4. Ultramicroelectrode response vs. potential applied for the injection of 1 μl of a solution of 30% cyclohexanone in triethylamine. (□) triethylamine response.

injection port temperature was 170°C, and the column temperature was 110°C. The polarization curves of the two species are shown in Fig. 4. A discontinuity in the response for both compounds is observed at about +6.0 V. An analogous feature between +4.0 and +6.0 V was observed in other binary mixtures. The same phenomena was observed in previous work [1] in the polarization curve for methylamine at potentials greater than about +2.5 V. The discontinuity is avoided if the ultramicroelectrode is mounted in the glass tube with a glass or glass solder instead of epoxy resin. This result suggests that the discontinuity is due to oxidative breakdown of the epoxy seal. While the degradation in response is eliminated when pure glass seals are used, the glass mounted ultramicroelectrodes are generally less sensitive, and their sensitivity is not increased with th pretreatment methods to any appreciable extent; other methods of pretreatment are under investigation.

Figures 3 and 4 demonstrate that analysis of an injected mixture is possible from the voltammetric gas phase response; more complex mixtures can be similarly analyzed. Figure 5 shows a chromatogram obtained at the thermal conductivity detector and the ultramicroelectrode detector for an injection of 1 μl of a solution containing 12% acetone, 12% cyclohexanone, and 30% toluene in 0-xylene (v/v). The ultramicroelectrode was maintained at +3.0 V vs the palladium pseudoreference electrode. The injection port temperature was 170°C and the column at 90°C.

We have studied the conduction mechanism across the insulating area between the two electrodes. Table 3 shows the response of the ultramicroelectrode detector to 1 μl injections of a series of substituted benzenes. The temperature of the injection port in each case was 170°C and the temperature of the column was 120°C. The potential of the ultramicroelectrodes was maintained at +3.0 V. As the number of methyl groups on the benzene ring is increased, the ultramicroelectrode response



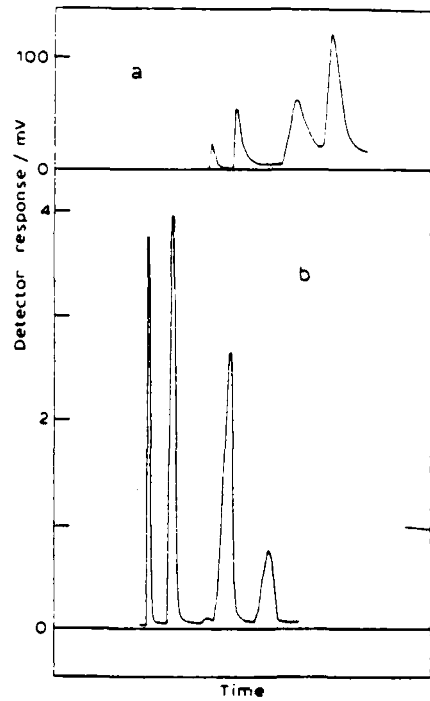


Fig. 5. (a) Ultramicroelectrode detector, (b) thermal conductivity detector responses after the injection of a solution conataining 30% toluene, 46% (v/v) o-xylene. The peaks from left to right correspond to acetone, touluene, o-xylene, and cyclohexanone, respectively. Applied potential was +3.0 V. The total time is 16 min.

Fig. 6. HPLC of a mixture of DNA and RNA bases. Lower chromatogram is the ultramicroelectrode response with potential difference of −3.0 V vs. the palladium secondary reference. Upper chromatogram is under the same conditions at −2.50 V. The injected concentrations of the bases were guanine: 11 pg ml^{-1}, adenine: 9 pg ml^{-1}, cytosine 10 pg ml^{-1} uracil: 19 pg ml^{-1}, and thymine 19 pg ml^{-1}. The peaks correspond to (a) guanine, (b) uracil, (c) cytosine, (d) adenine, and (e) thymine respectively.

decreases rapidly. This behavior is enhanced when the substituents on the benzene are ethyl groups. In general, methyl-substituted benzenes are anodically oxidized [15,16] to the cation radicals which lose a proton rapidly, to give the free radical. The free radical is oxidized further to and the following reactions lead ot a variety of products. In general, increasing the number of alkyl substituents on the benzene ring leads to a decrease in the oxidation potential of the hydrocarbon [17] and an increase in the stability of the cation radical. As a result, increasing the number of methyl groups leads to a decrease in the population of protons at the interface, although not in the total number of charged species. The response is observed to decrease with increased number of substituents (fewer free protons). Ethyl group

TABLE 3

Ultramicroelectrode detector response to methyl- and ethyl-substituted benzenes

Compound	Ultramicroelectrode response/pA [a]
Toluene (99%)	57.33
1,2-Dimethylbenzene (95%)	16.13
1,2,4-Trimethylbenzene (99%)	2.590
Pentamethylbenzene [b]	0.0000
Hexamethylbenzene [c]	0.0000
Ethylbenzene	0.0760
1,3,5-Triethylbenzene [c]	0.0000
Hexaethylbenzene [c]	0.0000

[a] Injection of 1 μ l.

[b] 7 *M* solution in acetone.

[c] Saturated acetone solution.

substitution leads to much greater stabilization of the cation radical (smaller population of free protons). As is seen in the table, the response drops markedly for ethyl substitution. The conclusion is that protons, and not necessarily other charged species produced in the redox reaction, are responsible for the ionic conductivity across the surface of the insulator separating the two electrodes.

The use of ultramicroelectrodes as detectors in resistive liquid solutions in high performance liquid chromatography is shown in Fig. 6. The ultramicroelectrode was not coated in this experiment. The DNA bases guanine, adenine, cytocine, uracil, and thymine were detected for a gradient elution of a mixture of the bases in a solution of acetonitrile in hexane (0 to 50%, v/v) in the chromatograph described in the experimental section. The small unlabeled peaks in the figure are due to impurities. The lower plot was the response of the ultramicroelectrode at −3.0 V while the upper plot was that obtained at −2.5 V. It is seen that the compound with the highest reduction potential, uracil, shows a much lower response at the lower potential, as expected. Further decrease in the potential results in the progressive and sequential disappearance of the responses in the order adenine, uracil, thymine, cytosine, and guanine, respectively, as expected from their reduction potentials. As the potential is made more positive, the response appear again, but with positive currents flowing. The sensitivity of the ultramicroelectrode detector is higher in the liquid system than in the gas phase experiments due to the increased conductivity of this medium.

It is clear that these types of detectors ar both quantity and species sensitive for chromatographic applications. Their use in a variety of new applications is evident.

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